Clinical, pathological and immunological aspects of periodontal disease

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The inflammatory and immune responses during the development and progression of periodontitis are reviewed. Susceptibility to periodontitis may be related to whether plasma cells predominate in the tissues of an individual, or a site, in response to the microbial insult from dental plaque. The tendency for an individual or site to form an extensive plasma cell infiltrate may indicate an inability to defend against periodontopathogenic bacteria and thus a predisposition to periodontitis. Selected pertinent areas of current interest in cellular and humoral immunology are considered within the periodontal context. These topical issues include (a) homing of immune and inflammatory cells to target tissues; (b) the local proliferation and synthetic activity of immune and inflammatory cells; (c) the cytokine profile of the inflammatory and immune cells; and (d) the immunoglobulin subclasses of locally produced antibodies.
☐ *Cellular immunity; cell proliferation; cell synthesis; chronic periodontitis; humoral immunity; plasma cells*

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The cellular and humoral immune responses function in both healthy and diseased periodontal tissues. During the immune and inflammatory reaction, fluid gathers at the sulcus and within the periodontal pocket, and within this fluid, specific host–microbe interactions occur. As a result, the sulcus/pocket fluid contains microbial, cellular, serum, inflammatory, immune, and tissue breakdown derived molecules. Sampling of this fluid is relatively easy and non-invasive; furthermore, periodontal tissue is easily accessible and obtainable from surgical procedures and from controlled clinical trials such as experimental gingivitis studies. The opportunity to collect disease-related tissues and fluids, which are not so easily obtained for many other human diseases, facilitates useful investigations of the immune and inflammatory processes during periodontal disease. Furthermore, surgery of the diseased periodontal tissues is a standard therapeutic intervention and provides biopsy tissue which is highly pertinent to the pathological investigations of the host response in periodontal disease. The inflammatory and immune responses in the gingival pocket of periodontal patients are thought to be initiated and perpetuated mainly by Gram-negative anaerobic bacilli and spirochetes. It is now evident that an antibody response of sufficient magnitude, specificity, and avidity can be protective in the periodontium. Aspects of both the cellular and humoral immune response are covered in this review. At the same time the value of the periodontal immune model in the study of a human disease process should become clear.

Homing–recruitment of specific inflammatory and immune cells to the periodontium

Although we have gathered much information about the immune response within the periodontium, our understanding is incomplete. For example, we do not know whether local plasma cells produce specific or non-specific antibodies against the periodontal microorganisms. We are beginning to understand some of the processes that have resulted in the accumulation of leukocytes in the periodontium. It now seems most likely that Langerhans cells and other antigen-presenting cells set up humoral immune responses within peripheral lymph nodes and that antibodies produced there are arriving at the local site to begin their function. However, this is clearly an inefficient process and a homing mechanism or a local proliferation of B cells into periodontally relevant plasma cells within the periodontium should be more efficient. There is now evidence that homing of both cellular and humoral immune cells is more pronounced in diseased periodontal tissues and that local proliferation seems to play a minor role (1, 2). Recruitment of leukocytes into areas of injury or infection is essential for effective host defense and the constant migration of immunocytes and other leukocytes to sites throughout the body allows the full repertoire of the immune system to protect the host from a variety of antigenic challenges. Migration of leukocytes into inflamed tissue results from the cytokine-induced expression of adhesion molecules on the surface of vascular endothelial cells. The changes in vascular adhesion molecule expression and numbers of infiltrating leukocytes during a 21-day experimental gingivitis episode were investigated by immunohistochemistry (3). ELAM-1 and ICAM-1 positive vessels and T cells and neutrophils were identified within gingival biopsies taken on days 0, 7, 14, and 21. Vascular endothelium expressed ELAM-1 and ICAM-1 both in clinically 'healthy' tissue (day 0) and in experimentally inflamed tissue (days 7 to 21). Positive vessels were found mainly in the connective tissue subjacent to the junctional epithelium, where the highest numbers of T cells and

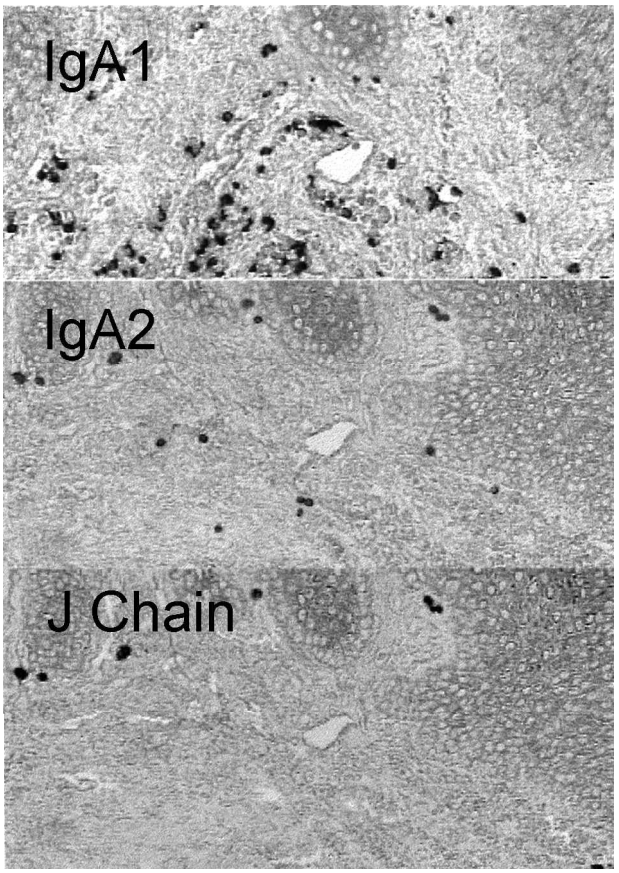

Fig. 1. The location of IgA-expressing plasma cells in adult periodontitis gingiva detected by in situ hybridization with oligonucleotide probes for IgA1mRNA, IgA2 mRNA, and J chain mRNA.

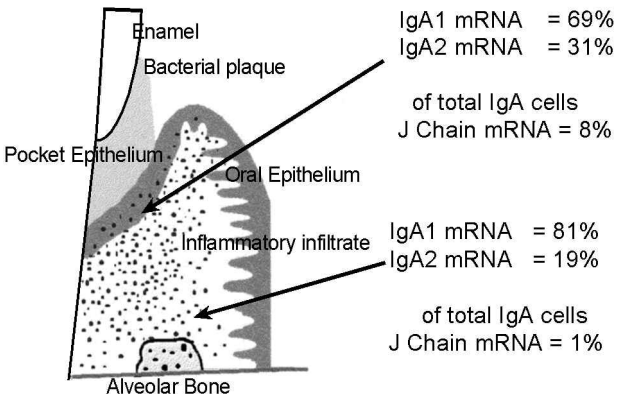

Fig. 2. Tissue localization of IgA-expressing plasma cells in periodontal tissue comparing expression proximal to the pocket epithelium with expression in the granulation tissue.

neutrophils were also seen. As the experimental gingivitis developed, the number of T cells or neutrophils in the different tissue regions did not change significantly, although the most intense vascular ICAM-1 and ELAM-1 staining redistributed to the connective tissue adjacent to the junctional epithelium. A gradient of ICAM-1 exists in the junctional epithelium, with the strongest staining on the sulcular aspect and this, along with the vascular expression of ELAM-1 and ICAM-1 in both clinically 'healthy' and inflamed tissue, suggests they are crucial processes which direct leukocyte migration into the tissues and towards the gingival sulcus. The importance of these mechanisms is highlighted by the rapid and severe periodontitis that characterizes leukocyte adhesion deficiency when no such recruitment takes place. The detection of secretory component and J-Chain expressing IgA2 positive plasma cells subjacent to the junctional and pocket epithelium (4) (Figs. 1, 2) prompted investigations into the possible involvement of the mucosal addressing cellular adhesion molecule (MAdCAM-1) and the vascular cell adhesion molecule (VCAM-1). Recent work in our laboratory has indicated that MAdCAM-1 is not involved in cell trafficking in the periodontium, while a role for VCAM-1 is implicated (D. F. Lappin, A. M. P. McGregor, D. F. Kinane. Unpublished observations, 2001).

Endothelial and leukocyte adhesion molecules play an important role in the recruitment of leukocytes and are necessary along with further processes in maintaining a population of relevant inflammatory and immune cells at specific sites of inflammation and disease. Nagai et al. (5) have shown an increase in $\gamma\delta$ T cell numbers in peripheral blood of periodontitis patients and it has been suggested that homing of these cells to the gingiva may occur during the disease process. This was supported by the finding that the proportion of $\gamma\delta$ T cells in gingival lesions shows a positive correlation with the size of the leukocyte infiltrate (6). Research in our laboratory (7) has demonstrated almost identical T cell gene rearrangement profiles between gingival biopsies from contralateral sites within the same individual, although the T cell profiles from skin regions were very different. Although these different T cell subsets could arise in the tissue by homing it is also possible that they could proliferate locally within certain tissues in response to local antigenic stimulation, thus giving rise to characteristic T cell clones for these regions. Recent reports from our laboratory show that the numbers of proliferating leukocytes in periodontal gingival and granulation tissue are low and are unlikely to account for a significant population of locally derived T cell clones (1, 2).

Immune cell proliferation and synthetic activity

Complex inflammatory and immune responses are involved in the progression of periodontitis. Tissue activity within the diseased periodontium comprises epithelial and connective tissue turnover and the cellular activity associated with the infiltrating inflammatory cells (8). Previous work has suggested that B cells and T cells

Table 1. The ratio of IL-4 to IL-2-expressing cells (Th2:Th1) in periodontitis granulation tissue from patients with early-onset periodontitis (EOP-GT) and chronic adult periodontitis (AP-GT) and gingiva (AP-Ging) from the patients with adult periodontitis

	EOP-GT		AP-GT		AP-Ging	
Ratio IL-4/IL-2	Mean	*s*	Mean	*s*	Mean	*s*
Antigen	21.3	8.2	19.0	10.2	30.8	8.7
mRNA	39.5	9.4	47.2	11.5	48.8	14.2

Each result represents the mean and standard deviation (*s*) of 12 sections.
Modified from Lappin et al. Clin Exp Immunol 2001;123:294–300.

accumulate in large numbers in the periodontal tissues and although we know a little about the cellular and synthetic activity and proliferation of these cells there is much about their functions in the disease process that is not clearly understood.

In our recent studies on periodontitis gingiva and granulation tissue we have demonstrated that the infiltrating lymphocytes were more likely to arise through selective homing than by local cell division (1, 2). Histone mRNA was detected with an oligonucleotide probe cocktail and Ki-67 antigen was detected with a specific murine monoclonal antibody. The synthesis of a short-lived histone protein, upregulated during the S-phase of the cell cycle (9), is coupled with DNA replication and the presence of its mRNA accurately defines proliferating cells within the S-phase of the cell cycle. Ki-67 antigen is synthesized in the nuclei of cells during G_1, S, G_2 and M phases of the cell cycle, but is absent from resting stage G_O and is a well-recognized nuclear proliferation marker (10–12). These studies demonstrated a lack of proliferating immune cells in the diseased periodontium, and suggest that the selective homing of pertinent immune cells is the most likely mechanism involved in the periodontal immune response.

While T cells are implicated in immunoglobulin synthesis in vitro (13–15), the results of these studies do not easily extrapolate to in vivo situations where complex interactions between a variety of infiltrated inflammatory cells occur. Furthermore, it may also be presumptive to assess the role of the different cell types and their interrelationships at inflammatory sites from limited observations of morphology and immunochemical analysis of cell surface markers. Immunohistochemical methods have been utilized to determine lymphocyte subsets (16–19) and the paradigms that 'periodontitis is a B cell lesion' and the 'immunoregulatory role of T cells in periodontitis' have been proposed following such studies (16, 20–22). One drawback of this sort of study is that the activity of the cells under investigation remains unknown.

Using in situ hybridization methodology we recently investigated the cellular activity in gingivitis and periodontitis lesions. The messenger RNA (mRNA) content of various cells in vivo was assessed with synthetic oligonucleotides (1, 2). We used poly-deoxyribothymidine (oligo d(T) to detect the poly-adenosine (poly-A) sequences of messenger RNA (mRNA). The number of total mRNA molecules and their species distribution is dependent on the level of cell activity, including the protein production of the cell, and its stage in the cell cycle (23, 24). We were able to show that the plasma cells were among the most active secretory cells in the gingiva. Fibroblasts and endothelial cells were moderately stained, whereas B and T lymphocytes were mostly weakly stained; some not stained at all.

These data support the view that B cells in the gingiva are long-lived cells. They probably possess the ability to migrate between the blood and lymph nodes to participate in the periodontal immune response. Our more recent findings indicate that B cells in the periodontal granulation tissues behave in a similar fashion (25). These observations are supported by the findings that: CD5 positive B cells are present in the gingiva in higher proportions than in blood (26); activated B cells are present in the periodontium (27); and CD5 positive B cells do not proliferate (28). There was no clustering of CD3 or of CD45RO positive cells in any of our sections of gingiva or granulation tissue. The above statements also appear to be true for T cells. While Reinhardt et al. (17, 18) have demonstrated that activation of T cells occurs in periodontitis, Longhurst et al. (29) have shown that T blasts are rare in transmission electron micrographs of periodontal tissue, although they have been observed in the infiltrated connective tissue adjacent to the junctional epithelium of slightly inflamed prepubertal gingiva (30).

Human T cell functions can be estimated by studying their cytokine profiles. Basically, three subsets of T-helper cells (CD4+) have been characterized by their cytokine profile (31, 32). Typical secretory products of Th1 cells are IL-2, IL-12, TNF-α, Interferon-γ, those of Th2 cells are IL-4, IL-5, IL-6, IL-10, IL-13, and Th3 cells are known to secrete TGF-α. In the past few years a large number of reports have investigated the role of Th1 and Th2 cells in the periodontium. While some conflicting reports have been published on the expression of IL-2 and IL-4 by T cells in the gingival, many recent studies on the immunology of periodontal disease support the concept that Th2 cells are more abundant than Th1 cells in the periodontitis lesions (33–38). Yet when the proteins or mRNA are prepared from cells isolated from crevicular fluid were measured, a pro-inflammatory and Th1 cytokine profile prevailed over a Th2 profile (39) (Table 1). However, this contradicts the earlier study of Pilon et

Table 2. Ratios of B-cells (CD 20+), T-cells (CD 3+), and macrophages (CD 68+) in the inflammatory infiltrate in periodontitis granulation tissue from patients with early-onset periodontitis (EOP-GT) and chronic adult periodontitis (AP-GT) and gingiva (AP-Ging) from the patients with adult periodontitis

	EOP-GT		AP-GT		AP-Ging	
Ratio	Q2	Q1–Q3	Q2	Q1–Q3	Q2	Q1–Q3
CD 20+	0.36	0.26–0.42	0.52	0.49–0.54	0.32	0.29–0.35
CD 3+	0.57	0.39–0.72	0.38	0.31–0.40	0.66	0.62–0.69
CD 68+	0.07	0.03–0.09	0.10	0.03–0.14	0.02	0.01–0.03

Each result represents the median (Q2) and interquartile range (Q1–Q3) of 12 sections.

al. (40), who showed that IL-2 levels were lower in crevicular fluid of periodontitis sites compared with healthy sites.

The relative importance of the Th1 and Th2 subsets in periodontal disease is poorly understood. Early reports have suggested that IL-2 mRNA was not expressed by T cells in the gingiva (41, 42); other studies suggest that gingival T cells express the protein (43, 44). Conflicting results of the early studies have also indicated that IL-4 was not expressed locally, since the mRNA encoding this protein was not detected by RT-PCR (41). However, it has since become clear that IL-4 can be detected immunochemically in gingival sections (45). Our recent observations (39) confirm the view that Th2 cells outnumber Th1 cells in periodontal lesions. We also confirm that typical products of both Th1 cells (IL-2, Interferon-γ, IL-15) and Th2 cells (IL-4, IL-6, IL-10) are detected in periodontitis gingiva and granulation tissue by detecting both the proteins and the mRNAs in the tissue sections (Table 1). In addition, we have confirmed the earlier observation (45) that the anti-inflammatory cytokine IL-10 is very widely expressed in periodontal tissue. The role pro-inflammatory and anti-inflammatory cytokines play in this disease process is still unclear. The concept that different cytokine profiles may characterize different developmental stages of periodontal disease has not been answered. That different populations of T cells in tissue lesions are associated with the gingivitis in the majority of people that does not progress into periodontitis and the gingivitis that ultimately leads to severe periodontitis in the minority of individuals is an important consideration that remains difficult to address.

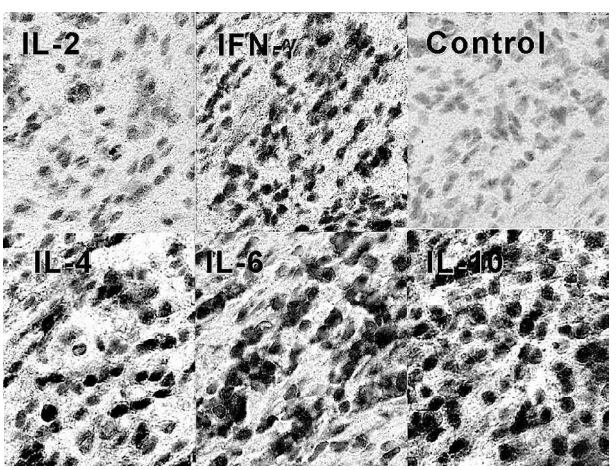

Fig. 3. The detection by immunohistochemistry of Th1 (IL-2, IFN-g) and Th2 (IL-4, IL-6, and IL-10) cytokines expressing cells in adult periodontitis granulation tissue.

We have observed increased numbers of T cells and a reduced number of macrophages in early-onset or aggressive periodontitis lesions when compared with chronic or adult periodontitis (24), but have not observed any differences in the cytokine profiles between these two diseases (39) (Table 2, Fig. 3). However, it is possible that differences might be present at earlier stages in the disease process and we must concede that only the later chronic phases of the disease have been investigated in our studies. Differences in the cell population are likely to be due to a large number of factors, including the genetic background of the individual, the presence of pathogenic and or absence of particular commensal microorganisms, the severity of the initial insult, and the duration and severity of the disease.

It is well known that there is a shift from a predominantly T cell to B cell lesion in the progression from gingivitis to periodontitis. It is interesting to speculate that a shift from cell-mediated immunity (Th1) to humoral immunity (Th2) occurs during the development of periodontal disease. At present the evidence is mainly circumstantial. It is apparent that in gingivitis T cells probably exceed cells of the B cell lineage, and when this progresses into periodontitis, B cells/plasma cells then predominate.

In conclusion, these studies support the hypothesis that the majority of specific leukocytes predominate in the periodontitis tissues through selective homing rather than by local proliferation.

Immunoglobulin subclasses in the periodontium

In addition to studies on the antibodies directed against bacteria, which will be discussed later, attention has focused on immunoglobulin (Ig) subclasses. The antibody production, especially of IgG and IgA, is considered to have a protective role in the pathogenesis of periodontal

Table 3. Proportions of plasma cells for each IgG subclass in diseased periodontal tissue from patients with early-onset periodontitis (EOP-GT) and chronic adult periodontitis (AP-GT) and gingiva (AP-Ging) from the patients with adult periodontitis

	Percentage of IgG mRNA expressing plasma cells							
	IgG1		IgG2		IgG3		IgG4	
Subclass	Mean	*s*	Mean	*s*	Mean	*s*	Mean	*s*
EOP-GT	63.5	7.7	24.7	6.6	4.1	2.7	7.7	2.7
AP-GT	64.9	5.8	23.9	6.8	4.0	2.5	8.2	2.8
AP-Ging	63.2	3.4	22.9	4.1	3.4	0.9	10.5	1.8
Serum conc	65.0		23.0		8.0		4.0	

Each result represents the mean and standard deviation (*s*) of 12 sections. Table modified from Kinane et al. Clin Exp Immunol 1999;115:534–41.

disease, but the precise mechanisms are still unclear. Although we are beginning to gain an understanding of the subject, there are certain parameters that need to be addressed; for example, what proportion of the antibodies in the gingival sulcus/pocket region comes from the systemic circulation and which antibodies are locally produced? In order to determine local IgG and IgA production, we investigated the human IgG and IgA subclass mRNA-bearing plasma cells within periodontal tissue by in situ hybridization using digoxigenin-labelled oligonucleotide probes in gingival samples from periodontitis patients (46). In addition, the concentrations of IgG and IgA subclass proteins and digested IgA1 Fab portions were measured in the pocket fluid that corresponded to the sites from which the tissues were taken (47). IgG1 mRNA-expressing cells were predominant (mean 63%) and IgG2 mRNA-expressing cells were present at around 23% of total IgG plasma cells and IgG3 and IgG4 were present to a lesser extent (3% and 10%, respectively) (Table 3). Similar proportions of IgG subclass proteins were detected in pocket fluid, which were also consistent with typical serum levels. In terms of IgA expression, IgA1 mRNA-positive cells were the greater (mean 65.1%, $P < 0.001$). In contrast, IgA2 protein in the pocket fluid samples were present at higher concentrations than IgA1 ($P < 0.001$) and IgA1 Fab fragments were detected more than intact IgA1. There was good correlation between the amounts of IgG subclass proteins in pocket fluid and the number of IgG subclass mRNA-positive cells in the same sites, but not between IgA subclass proteins and the number of IgA subclass mRNA-positive cells.

These data suggest that IgG and IgA subclass proteins can be produced locally in the periodontium and, in addition to serum-derived immunoglobulin, contribute to the antibody levels found in the gingival pocket or crevice. Furthermore, IgA1 Fab fragments were detected in pocket fluid and it is suggested that some of the secreted IgA1 protein may be digested by periodontal pathogens within the pocket fluid.

Further studies have confirmed the local expression of immunoglobulins in periodontal lesions. IgM, and IgG and IgA subclass proteins and J-chain can be locally produced in periodontitis granulation tissues. IgG1 mRNA-expressing cells were predominant in the granulation tissues as in the gingiva, constituting approximately 65% of the total IgG expressing plasma cells. However, there was a significantly greater proportion of IgA expressing plasma cells in the gingiva compared to the granulation tissue ($P < 0.01$). The majority of the IgA expressing plasma cells were IgA1, but a greater proportion expressed IgA2 mRNA and J chain mRNA in the gingival tissues (30.5% and 7.5%, respectively) than in the periodontal granulation tissues (19% and 0%–4%, respectively). The J chain or dimeric IgA2 expressing plasma cells were located adjacent to the epithelium, suggesting that this tissue demonstrates features consistent with a mucosal immune response (Fig. 1). The presence of the secretory component in gingival and junctional epithelial cells showed that the periodontal epithelium shares features with mucosal epithelium. In contrast, deeper tissues have more plasma cells that express IgM, and less expressing IgA, a response that appears more akin to the systemic immune response. This suggests that immune mechanisms involved in the pathogenesis of periodontitis may involve features of both the mucosal and systemic immune systems and that this is dependent on tissue location (4). The increased proportion of IgA2 in crevicular fluid might be due to the presence of an active secretory process for dimeric IgA2 in the pocket and junctional epithelial cells.

There is a possibility that a protective role afforded by the secretory immunoglobulin (dimeric IgA2) is supplanted by a much greater and inadvertently more destructive IgG response. This process may be indicative of an immune response, which in certain individuals has failed to neutralize the antigenic challenge mounted by particular oral pathogens. The converse is also possible that these features of a mucosal type immune response are inappropriately added to a systemic response that has failed to destroy the immune targets.

Conclusion

These studies on selected aspects of the humoral and cellular immune response in periodontal disease indicate that: (a) homing of relevant immune cells takes place within the periodontium; (b) although Th2 cells outnumber the Th1 cells in chronic periodontal lesions we are

still unsure as to the significance of these findings; (c) plasma cells are among the most active secretory cells in the gingiva; (d) immunoglobulin subclasses are similar between blood and the gingival pocket fluid for IgG but not for IgA; (e) the overriding features of periodontal histopathology are those of a systemic immune response, although mucosal immune responses are present which could in fact demonstrate a dysregulation of response due to the overwhelming disease process; (f) we are left with the hypothesis that an individual's ability to mount a specific antibody response to periodontopathogenic organisms may indicate their susceptibility to the disease and their likely response to treatment.

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